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Regulationseffekte während der Antikörpersynthese bei Parabionten

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Regulation of Antibody-Formation in Parabiosis

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Summary. This work was designed to show whether antibodies can influence the immune response *after* sensitization. — NMRI-mice were sensitized with sheep red blood cells (SRBC) and joined by parabiosis. After different intervals the immune response was examined by detection of single antibody-forming cells using the plaque-technique. For demonstration of a possible transfer of SRBC or antibody-producing cells only one parabiont was sensitized.

The results show a mutual influence of the immune response of both parabionts. Because there is no transfer of SRBC or antibody-producing cells through the communication the influence is due to passing antibodies. Those antibodies inhibit the immune response more when animals are sensitized few days before parabiosis. The suppression of the antibody-formation in animals sensitized before parabiosis is an indication for a direct interaction of antibodies with cells producing them.

Key words: Cellular Antibody-Formation — Parabiosis — Plaque-Technique — Regulation of the Immune Response.

Zusammenfassung. Die Untersuchungen sollten zeigen, inwieweit bei Parabionten die Immunantwort gegenseitig beeinflusst werden kann. — NMRI-Mäuse wurden zu unterschiedlichen Zeitpunkten mit Hammelerythrocyten (HE) sensibilisiert und parabiontisch vereinigt. Durch Auszählen Antikörper-bildender Milzzellen wurde die Stärke der Immunantwort bestimmt, wobei die Plaque-Technik angewandt wurde. Durch Sensibilisieren eines einzigen Partners vor der Parabiose sollte gezeigt werden, ob einzelne Antikörper-bildende Zellen oder HE durch die Anastomose übertreten.

Die Ergebnisse zeigen eine gegenseitige Beeinflussung der Immunantwort. Da einzelne Antikörper-bildende Zellen oder HE nicht übertreten, ist dieser Effekt allein zirkulierenden Antikörpern zuzuschreiben. Sie führen zu einer Hemmung der Immunantwort insbesondere bei Tieren, die kurz vor der Parabiose sensibilisiert worden sind. Die Ergebnisse bestätigen das Vorliegen eines Rückkoppelungsmechanismus und beweisen darüber hinaus, daß die Unterdrückung durch direktes Einwirken auf die Antikörper-bildenden Zellen erfolgt.

Schlüsselwörter: Celluläre Antikörperbildung — Parabiose — Immunregulation.

A feedback system has been revealed during the investigation of the possible suppression of the immune response by passive transfer of specific antibody [2, 4, 7]. The mode of action is thought to be a removal

of antigenic determinants by the antibody. Therefore antibodies with high affinity will be more efficient inhibitors [6]. Moreover, a direct action of the antibodies on the producing cells has been suggested [5]. For further characterization of the feedback mechanism parabiosis has been established to demonstrate an influence on the immune response after sensitization. Jerne's plaque-technique has been used to detect single antibody producing cells for quantitative determination of the immune response.

Material and Methods

Female mice of the inbred-strain NMRI have been used. The age was about 3 months, the mean weight was 35 g. 4 to 8 animals were kept in one box. When fixed together one pair of parabionts were kept in one box. The animals got pellets and water ad libitum.

Sensitization. The animals were sensitized by intraperitoneal application of 4×10^8 sheep red blood cells (SRBC) washed in buffered saline.

Parabiosis. The animals were anesthetized by i.p. application of pentobarbital and restrained on a special table. After shaving of the hairs and disinfection of the skin a lateral abdominal incision of 2 cm length was performed. The adapted layers were sutured separately. After this operation the animals were immobilized in a plaster cast. At the end of the parabiosis the permeability of the communication was proved by i.p. instillation of methylen-blue.

Demonstration of Cellular Antibody-Formation. The animals were sacrificed by cervical dislocation. The spleen was removed and separated from fat and connective tissue. To demonstrate single antibody-forming cells the plaque technique [1, 3] was used: the spleen was pressed through a stainless steel mesh and the cells were washed in buffered saline. 10^6 spleen cells were dispersed in gel among 4×10^8 SRBC. Adding complement after incubation at 34°C for 90 min. 19S-producing "direct plaque forming cells" (DPFC) were marked by local hemolysis in gel (= plaques) around those cells. In an other series anti mouse globulin was added as "developing serum" before complement. After incubation 7S-antibody producing "indirect plaque forming cells" (IPFC) could be identified.

Influence of Parabiosis on the Immune Response and Passage of PFC. To examine the influence of the operation and of the parabiosis on the immune response, sensitized animals were brought in parabiosis with unsensitized animals. These experiments should also demonstrate a possible transfer of PFC from the sensitized into the unsensitized partner.

Series I. Partner A was sensitized 2 days before parabiosis, partner B was not sensitized. Plaque-technique was performed in both parabionts at the same time, i.e. 4 pairs of parabionts were examined 1 day after parabiosis, 4 pairs of parabionts 8 days after parabiosis had been established.

Series II. Partner A was sensitized 5 days before parabiosis, partner B was not sensitized. Plaque-technique was performed in both parabionts at the same time, i.e. 4 pairs of parabionts were examined 1 day after parabiosis, 4 pairs of parabionts 8 days after parabiosis had been established.

Series III. Partner A was sensitized 10 days before parabiosis, partner B was not sensitized. Plaque-technique was performed in both parabionts at the same time, i.e. 4 pairs of parabionts were examined 1 day after parabiosis, 4 pairs of parabionts 8 days after parabiosis had been established.



Table 1. *Experimental plan and results of the series I, II and III*

<i>Series I</i>		T_1		T_2	
		DPFC	IPFC	DPFC	IPFC
Animal A	S	P T_1 T_2		A	146 54 20 7
Animal B		P T_1 T_2		B	2.75 2.0 — —
Animal C	S	T_1	T_2	C	105 45 12 10
Days	0	2 3	10		54 275 7 27

<i>Series II</i>		T_1		T_2	
		DPFC	IPFC	DPFC	IPFC
Animal A	S	P T_1 T_2		A	286 234 13 11
Animal B		P T_1 T_2		B	6.75 4.5 — —
Animal C	S	T_1	T_2	C	218 194 11 12
Days	0	5 6	13		6.75 156 — 11

<i>Series III</i>		T_1		T_2	
		DPFC	IPFC	DPFC	IPFC
Animal A	S	P T_1 T_2		A	56 357 5 27
Animal B		P T_1 T_2		B	5.75 0.25 — —
Animal C	S	T_1	T_2	C	28 250 3 18
Days	0	10 11	18		5.0 32 — 3

The table reports the mean values and standard errors (placed below) of DPFC and IPFC counted in 10^6 spleen cells of animals A, B and C, separately examined at T_1 (1 day after begin of parabiosis) and T_2 (8 days after begin of parabiosis). Standard errors are not calculated, when the counts of PFC are within the limit of the background.

S = Sensitization. P = Begin of parabiosis. T_1 , T_2 = Plaque-technique.

Table 2. *Experimental plan and results of the series IV*

Animal A	S			P	T ₁	T ₂
Animal B		S		P	T ₁	T ₂
Animal A _c	S				T ₁	T ₁
Animal B _c		S			T ₁	T ₂
Days	0	24	28	32	34	

	T ₁		T ₂	
	DPFC	IPFC	DPFC	IPFC
A	4.5 —	13 2.74	3.5 —	15.5 2.66
B	27 3	45 3	11.5 —	32 3
A _c	1.25 —	8.5 —	1.5 —	13.75 2.23
B _c	99 6	224 12	36 4	143 13
A : A _c	2.15 : 1	1.53 : 1	2.3 : 1	1.12 : 1
B : B _c	1 : 3.7	1 : 5	1 : 3.1	1 : 4.5

The mean values and standard errors of DPFC and IPFC counted in 10^6 spleen cells of the animals A, B, A_c and B_c are summarized. The quotients A : A_c and B : B_c demonstrate the influence of antibodies passing through the communication on the immune response of the animals A and B. The more A_c or B_c exceed A or B, the more efficient is the suppression of the immune response in A and B.

The standard error (placed below the mean values) is not calculated when the counts of PFC are within the limit of the background.

S = Sensitization. P = Begin of Parabiosis. T₁, T₂ = Plaque-technique.

Controls. At the same day as the animals A solitary animals, called C, were sensitized with the same suspension of SRBC. Plaque-technique was performed at the same day as in both parabionts A and B.

The experimental plan is shown in Table 1.

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Experiments were performed with partners sensitized at different intervals before parabiosis.

Series IV. Partner A was sensitized 28 days before parabiosis, partner B was sensitized 4 days before parabiosis. Plaque-technique was performed in 4 pairs of

Table 3. *Experimental plan and results of the series V*

Animal A	S	<table><tr><td>P</td><td>T₁</td><td>T₂</td></tr><tr><td>P</td><td>T₁</td><td>T₂</td></tr></table>					P	T ₁	T ₂	P	T ₁	T ₂
P	T ₁	T ₂										
P	T ₁	T ₂										
Animal B		S										
Animal A _c	S	T ₁ T ₂										
Animal B _c		S	T ₁ T ₂									
Days	0	26	28	32	34							

	T ₁		T ₂	
	DPFC	IPFC	DPFC	IPFC
A	1.0 —	6.25 —	1.0 —	7.0 —
B	11.25 1.28	23 3	21 3	57 4
A _c	1.0 —	7.25 —	1.5 —	13.75 2.25
B _c	47 5	63 11	83 5	145 7
A : A _c	1 : 1	1 : 1.15	1 : 1.5	1 : 1.96
B : B _c	1 : 4.2	1 : 2.6	1 : 3.95	1 : 2.5

The mean values and standard errors of DPFC and IPFC counted in 10^6 spleen cells of the animals A, B, A_c and B_c are summarized. The quotients A : A_c and B : B_c demonstrate the influence of antibodies passing through the communication on the immune response of the animals A and B. The more A_c or B_c exceed A or B, the more efficient is the suppression of the immune response in A and B.

The standard error (placed below the mean value) is not calculated when the counts of PFC are within the limit of the background.

S = Sensitization. P = Begin of Parabiosis. T₁, T₂ = Plaque-technique.

parabionts 4 days after parabiosis, in 4 pairs of parabionts 6 days after parabiosis had been established.

Controls were animals A_c and B_c that were sensitized with the same suspension of SRBC at the same day as the animals A and B. Plaque-technique was performed after the same interval as in the animals A and B.

The experimental plan is shown in Table 2.

Series V. Partner A was sensitized 28 days before parabiosis, partner B was sensitized 2 days before parabiosis. Plaque-technique was performed in 4 pairs of parabionts 4 days after parabiosis, in 4 pairs of parabionts 6 days after parabiosis had been established.

Controls were animals A_c and B_c that were sensitized with the same suspension of SRBC at the same day as the animals A and B. Plaque-technique was performed after the same interval as in the animals A and B.

The experimental plan is shown in Table 3.

It should be stated that all animals were examined separately.

Results

The parabionts showed no malaise. They took sufficient food and water. A loss of weight was not seen. When the abdomen was opened no pathological findings could be demonstrated. Some sensitized animals had a slightly enlarged spleen. The communication was functional in all experiments.

Series I. Sensitized animals A showed a good immune response. Moore IPFC could be found when plaque-technique was performed at a greater interval after parabiosis. The counts of PFC in unsensitized animals were not greater than the "background".

In controls C the counts of PFC were fewer than in sensitized animals A.

Series II. Sensitized animals A showed a good immune response. The greater the interval between sensitization and parabiosis was, the more IPFC could be found in relation to DPFC. Unsensitized partners B had not more PFC than the background. Controls C had fewer counts of PFC than sensitized animals A.

Series III. According to the large interval between the sensitization and plaque-technique the counts of IPFC were much greater than the counts of DPFC. Unsensitized animals B had counts of PFC within the limit of the background. In control animals C fewer PFC were found than in animals A.

The results of the series I, II and III are shown in Table 1.

Series IV. All parabionts showed an immune response. The counts of PFC were suppressed in the animals B in comparison to the counts in the controls B_c .

Series V. All parabionts showed an immune response. The counts of PFC were suppressed in the animals B in comparison to the counts of PFC in the controls B_c .

Because of the large interval between sensitization and plaque-technique in the animals A and A_c of the series IV and V the counts of PFC are very low. Therefore the relation of the counts of the animals A to the counts of the animals A_c is inconstant.

The results of the series IV and V are summarized in Tables 2 and 3.

Discussion

An interaction between both parabionts regarding the immune response was clearly demonstrated. The preparing series I, II and III demonstrated that there is no remarkable passage of PFC from one parabiont to the other. It may be that a small number of PFC passed through the communication but did not migrate into the spleen. There is also no sign of induction of immune response in the unsensitized animals by a permeating factor. Moreover, the lack of PFC in unsensitized animals demonstrates that no SRBC have passed through the communication into the unsensitized partner.

The increase of PFC during the parabiosis is due to an unspecific effect resulting from the operative trauma and the postoperative stress. There was no sign of a specific increase due to incompatibility, for previous experiments had shown that parabiosis for more than 30 days does not cause any activation of the RHS.

The interaction on the immune response therefore is caused by antibodies passing through the communication. The influence is more pronounced when the sensitization was performed few days before parabiosis. Thus a regulating influence on the antibody-formation in the animals A, sensitized 28 days before parabiosis, cannot be demonstrated. In contrast, the counts of PFC are markedly decreased in the partner B, sensitized only 2 or 4 days before parabiosis. This can be explained by a stronger interaction on the immune response due to antibodies with high affinity. Because of the increasing affinity of circulating antibodies during the immune response "late" antibodies of the animals A have a high affinity in comparison to "early" antibodies of the animals B. Therefore the influence on the immune response is more distinct in the animals B than in the animals A. Another important factor is the greater efficiency to suppress the immune response when antibodies are transferred immediately after the sensitization.

The antibody-formation of the series IV and V is inhibited in a different manner. The counts of DPFC decreased more than the counts of IPFC when sensitization was performed 2 days before parabiosis. Other results are found when sensitization is performed 4 days before parabiosis. This different interaction is caused by two factors: a) any disorder is more easily recognized when the influenced reaction is at the peak. Because the 19S-immunoglobulin synthesis is started before the synthesis of 7S-immunoglobulins, the suppression of DPFC was more pronounced in animals sensitized 2 days before parabiosis, whereas the suppression of IPFC was demonstrated most impressively in animals sensitized 4 days before parabiosis. b) IgG does inhibit the production of IgM and IgG (negative feedback), whereas IgM forces the production

of IgG (positive feedback). Thus the inhibition caused by the passing "late" antibodies is intensified by own antibodies. This means a high suppression of the IgG-producing IPFC and a low suppression of the IgM-producing DPFC as it could be demonstrated in these experiments.

The counts of PFC in the animals A and A_c were very low. This is due to the large interval between sensitization and plaque-technique. According to this fact animals A had partially more and partially less PFC than animals A_c. This demonstrates that the immune response in the animals A was not suppressed.

The results agree with the known facts of a regulation of the immune response. It is much important, however, that the inhibition of antibody-formation is possible *after* sensitization. At this point SRBC are no more available. Therefore there is no binding of antigen possible representing a "peripheric" mechanism of the feedback system. The experiments evidence a direct or "central" influence of antibodies with cells that are producing them.

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L E B E N S L A U F

Am 9. September 1944 wurde ich in Mügeln/Sachsen geboren, als fünftes Kind des Bürgermeisters Herbert Thümmel und seiner Ehefrau Marianne, geb. Kießig.

Dort begann ich 1951 meine Schulausbildung mit dem Eintritt in die Volksschule. Im Oktober 1953 übersiedelten wir nach Landshut, wo ich 1955 in die damalige Oberrealschule eintrat. An derselben Schule bestand ich im Sommer 1965 das Abitur.

Von Oktober 1965 bis September 1966 leistete ich dann im Sanitätsdienst der Bundeswehr meinen verkürzten, vorzeitigen Wehrdienst ab.

Seit dem Wintersemester 1966/67 studierte ich an der Friedrich-Alexander-Universität Erlangen-Nürnberg Medizin. Das Vorphysikum legte ich im Sommersemester 1967 ab, das Physikum am Ende des Wintersemesters 1968/69.

Das Medizinstudium beendete ich mit dem Staatsexamen am 28. Juli 1972.

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